STUDIES ON THE LIFE-HISTORY OF ALLOCREADIUM ALLONEOTENICUM SP. NOV. (ALLOCREADIIDAE–TREMATODA)

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The systematics of the trematode genus *Allocreadium* has been confused because of studies which reported two cercarial types from members of the genus. The cercariae which have been reported are not only morphologically dissimilar, but the molluscan hosts include both sphaeriid clams (Looss, 1894; Dollfus, 1949; and Peters, 1957) and prosobranch snails (Seitner, 1951).

Looss (1894) and Dollfus (1949) described an ophthalmoxiphidiocercaria developing from rediae in sphaeriid bivalves as the cercarial stage of the type species *A. isoporum* (Looss 1894). Peters (1957) also described a similar ophthalmoxiphidiocercaria from rediae developing in sphaeriid bivalves as the cercarial stage of *A. neotenicum* Peters 1956. These three authors did not demonstrate the life cycles of the parasites experimentally but based their conclusions on morphological similarities of the cercariae and adults. The ecological evidence presented in each case supported their conclusions.

Seitner (1951) described the larval forms of *A. ictaluri* Pearse 1924, from *Pleurocera acuta* (Say), a prosobranch gastropod. In this case, the cercaria was described as a gymnocephalous biocellate form, bearing setae in symmetrically arranged papillae on the body and tail. Seitner pointed out that it is extremely unlikely that species in the same genus would be morphologically different and that they would have such widely different molluscan hosts. Since Seitner's work was supported by experimental evidence he correctly regarded the earlier conclusions of Looss and Dollfus as inconclusive. Peters (1957) pointed out, however, that there are several reasons to question whether the cercaria described by Seitner is actually the larva of *A. ictaluri.* Peters further showed that morphological and ecological data tended to prove that Seitner was probably dealing with the larval stages of *Skrjabinopsolus manteri* (Cable 1952), a lepocreadioid, instead of *A. ictaluri.*

Mathias (1937) reported on the life-history of *Allocreadium angusticolle* (Hausmann) but this trematode has since been placed in the genus *Coitocaecum* by Dollfus (1949).

Evidence from controlled experiments in the present study supports the morphological and ecological observations of Looss, Dollfus and Peters, since the molluscan hosts are sphaeriid clams which liberate ophthalmoxiphidiocercariae. These penetrate into caddis fly larvae and become precociously mature in the haemocoel of these hosts.

Methods and Materials

Materials used in this study were obtained from the clam, *Pisidium abditum* Haldeman, and from caddis fly larvae belonging to the genus *Limnéphilus* collected

at two localities near Falmouth on Cape Cod, Mass. One collecting area was a spring-fed pond draining into the Coonamessett River just off Sandwich Road and the other was an extensive cranberry-ditch area along the Quashnet River just off Highway 28. Other caddis fly larvae and various beetle larvae and adult beetles occurring in the type localities were never infected in nature and were refractive to experimental infection. Living material, from both fingernail clams and caddis fly larvae, was used for the study of the excretory system and other morphological details.

A caddis fly larva harboring adult flukes (Fig. 1) could be determined by forcing the larva partially out of the case and observing the appendages and abdominal area for the presence of the eggs of the parasite. The infected larvae were characteristically darker brown than uninfected ones because of the presence of the eggs. If eggs were present in the larvae, at least one adult fluke was present. In a few cases, the fluke had degenerated so that they appeared as a blackish, internally amorphous mass which, however, still retained the characteristic external shape of the worm. Various degenerative stages of the flukes were observed to form an uninterrupted series so that it was evident that this was not an isolated or abnormal occurrence. These stages were more often found in caddis fly larvae harboring many flukes. (Some contained up to 25 worms.)

Worms and eggs were obtained by carefully detaching the head from the larvae, dissecting the last two segments from the abdomen and withdrawing the intestine, thus allowing space for the escape of the flukes from the haemocoel.

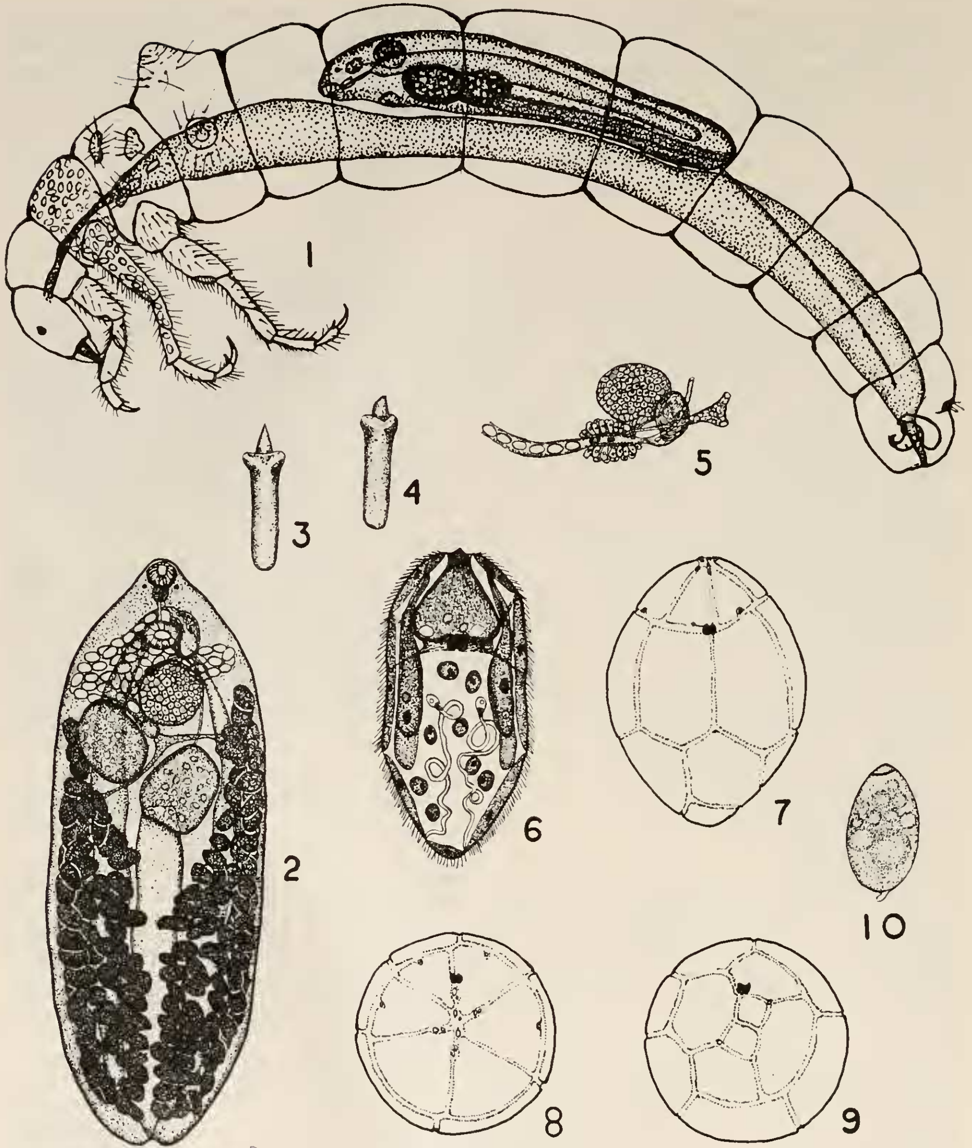
Specimens were fixed by squirting them into Gilson's fluid at 60° C. Whole mounts were stained with Semichon's aceto-carmine and Harris' haemotoxylin. Sections of uninfected and infected caddis fly larvae with worms *in situ* were stained with Delafield's haematoxylin.

Cercariae were studied alive with the aid of aqueous vital stains such as neutral red, orange G, brilliant cresyl blue, and Nile blue sulphate and also as fixed and stained specimens.

Two methods were used to obtain miracidia for study. One was to wash out as many eggs as possible from dissected material, then to remove the remains of the larvae and wash the eggs. Such "clean" eggs did not hatch even when filtered water from finger bowls containing an abundance of dead leaves and other organic material was substituted for stream water. When numerous miracidia could be observed actively moving within the eggs, small snails, *Aplexa hypnorum* (L.), from Coonamessett pool were added. The snails readily ingested the eggs and as eggs appeared in the feces of the snail, the miracidia began to hatch. This process was observed under the dissecting microscope. The other method of obtaining miracidia, which gave equal results as far as numbers of miracidia was concerned, was to dissect the caddis fly larvae but to leave the remains in the container with the eggs and to add filtered water rich in organic acids. This method had the disadvantage of encouraging bacterial and protozoan growth.

Miracidia obtained by these two methods were studied, alive by the use of vital stains, and also as fixed and stained specimens. For the study of the epidermal plates, the miracidia were impregnated using the silver nitrate method of Goodchild (1948) and mounted in glycerine jelly.

All measurements used in this study are in millimeters. Length is given first, followed by width.



Explanation of Plate I

Figure 1. *Limnéphilus* sp. (10 mm. in length); lateral view with *Allocreadium alloneotenicum* adult in haemocoel. Eggs shown only in middle thoracic segment. Entire figure diagrammatic.

Figure 2. Adult (3.5 × 1.3 mm.), ventral view.

Figure 3. Cercarial stylet (0.0238 mm. in length).

Figure 4. Worn stylet of adult (0.023 mm. in length).

Figure 5. Female complex, lateral view.

Figure 6. Miracidium (0.095 × 0.045 mm.), dorsal view.

Figure 7. Miracidial epidermal plates (0.061 × 0.044 mm.), dorsal view.

Descriptions of Stages in the Life-Cycle

Allocreadium alloneotenicum n. sp.: The specific name *alloneotenicum* ("another" neotenicum) was chosen because of the similarity in neotenous development to *A. neotenicum* Peters 1957.

Host: The caddis fly larva *Limnéphilus* sp.
Site: Haemocoel.
Incidence: 40 of 120 larvae (30%).
Type locality: Coonamessett River, Barnstable County, Cape Cod, Massachusetts, U. S. A.
Type specimens: A type and paratype will be deposited in the Helminthological Collection of the United States National Museum.

Adult (Fig. 2):

Body elliptical, 1.38–4.42 × 0.59–1.41 mm., thick but slightly flattened dorsoventrally, anterior end bluntly pointed; usually with posterior indentation at excretory pore; cuticle smooth. Well developed eyespots, located at level of posterior margin of oral sucker, present in all stages of development. Oral sucker subterminal 0.12–0.20 × 0.08–0.19 mm. (average 0.16 × 0.14 mm.). Stylet present (Fig. 4), imbedded in dorsal lip of oral sucker in relatively same position as in cercaria at an angle of 45°–60° from the longitudinal axis of the body. Ventral sucker 0.13–0.22 × 0.10–0.22 mm., (average 0.175 × 0.156 mm.), in anterior one-sixth or one-seventh of body. Prepharynx short, usually not evident but distinguishable in sections. Pharynx spherical, 0.06–0.11 mm. in diameter, posterio-dorsal to oral sucker. Esophagus as long as, to twice as long as, pharynx; intestinal bifurcation at level of anterior edge of ventral sucker, caeca simple, somewhat inflated, extending dorsally almost to posterior end of body. Excretory bladder elongate, sac-shaped with well defined lumen, extending anteriorly to middle of posterior testis. Excretory pore terminal within indentation at posterior end of body. Main excretory tubules extend anterio-laterad from bladder in a somewhat tortuous pattern, but without recurrent loop to level of mid-anterior testis where each receives an anterior and posterior secondary tubule. Each secondary tubule drains three groups of flame cells, exhibiting considerable variation both in numbers and position. Apparent flame cell formula is $2\,[(6+6+6)+(6+6+6)]$.

Ovary oval, 0.21–0.37 × 0.19–0.46 mm. (average 0.28 × 0.31 mm.), posterior to ventral sucker, sometimes overlapping the latter. Oviduct extends mediad a short distance, receives the common duct of receptaculum seminis and Lauer's canal (Fig. 5), then turns anteriad. Receptaculum seminis variable in shape, usually about one-fourth size of ovary. Sinuous Lauer's canal opens dorsally, posterio-median to ovary. Oviduct extends to form the oötype which is surrounded by a prominent Mehlis' gland; from oötype the uterus extends anteriorly composed of few loops, usually confined to area on right side of body bounded posteriorly by anterior testis and medially by ovary, but occasionally overlapping these struc-

Figure 8. Miracidial epidermal plates (0.044 mm. in diameter), anterior view.
Figure 9. Miracidial epidermal plates (0.044 mm. in diameter), posterior view.
Figure 10. Egg (0.096 × 0.056 mm.), lateral view.

tures. Uterus sometimes with a loop or two anterior to the ovary on left side of body. Metraterm present, opening into genital pore. Shallow genital pore ventromedian, near level of pharynx.

Vitelline follicles extend posteriad from anterior edge of ovary, more or less confluent in post-testicular half of body on ventral side, reaching almost to posterior end of body. Right and left vitelline ducts unite to form the vitelline reservoir in the angular space between the testes and ovary.

Testes oval or irregular in outline, never lobed, subequal, anterior testis, 0.19–0.46 × 0.24–0.44 mm., (average 0.29 × 0.35 mm.); posterior testis, 0.19–0.51 × 0.19–0.59 mm., (average 0.31 × 0.39 mm.). Testes diagonal, contiguous or separated by a short distance, ventral in position and confined to anterior one half of body. Vasa efferentia arise on anterior margin of testes, proceed anteriorly and join at posterior end of cirrus sac to form a very short vas deferens which enters cirrus sac forming a vesicula seminalis. Cirrus sac, 0.09–0.22 × 0.12–0.34 mm. (average 0.14 × 0.22 mm.), median or submedian to left of mid-line, between pharynx and ventral sucker; vesicula seminalis convoluted, pars prostatica tubular, prostate cells numerous and well developed.

Eggs number up to 45, measuring 0.092–0.108 × 0.055–0.060 mm. (average 0.0975 × 0.0577 mm.) with a small antopercular knob (Fig. 10), shell thin, light golden brown.

Miracidium (Fig. 6):

When first laid, the eggs are segmented. Development of the eggs is extremely variable. Within 48 hours, motile miracidia can be observed in some eggs, in others movement of miracidia can not be observed for several days. Upon hatching, the miracidium swims in a slightly zig-zag path, rotating slowly. Miracidia positively phototactic, converging either on light side of shallow dish or on opposite side where light rays are concentrated after passage through the water. Body of miracidium either elongate or pear-shaped when swimming, terrebratorium sometimes protruded. Miracidia were infective to both laboratory-reared young *Pisidium abditum* and *Musculium partumeium* (Say). No attraction to clam hosts was observed but infections were present within 24 hours after clams were added to the container with miracidia.

In using the Goodchild (1948) modification of Lynch's (1933) silver nitrate method of delineating epidermal plates, it was found that if the 1.0% silver nitrate solution was warmed slightly, there was less distortion of the miracidium. Silver nitrate-treated miracidia measured 0.061 × 0.044 mm.

The epidermal cell formula is 6–6–4–2 (Figs. 7, 8, 9). The same formula was observed by Peters (1957) for *A. neotenicum*. The miracidia of both these neotenous forms are quite similar, agreeing closely in most respects. The anterior and second tiers consist of two ventro-lateral, two dorso-lateral and two lateral cells lined up essentially end-to-end. The four cells in the next tier are arranged with a ventro-lateral and a dorso-lateral cell on each side, while the posterior tier consists of a dorsal and a ventral cell. The conspicuous double eyespots are situated dorsally about one-third of the length from the anterior end in living miracidia. The nervous system surrounds the eyespots and sends branches laterally to sensory pores and posterior branches diagonally to the middle of the lateral ciliary plates in the second tier. The anterior fourth of the body is occupied by the apical

gland which is without a stylet, and with 3–4 nuclei along its posterior margin. Granular structures representing the "penetration" glands of other authors are difficult to see clearly. They occupy most of the lateral portions of the miracidium. Observations of miracidial penetration clearly show four discrete unicellular glands. Two flame cells, usually not at same level, lie near the middle of the miracidium. Five to nine germinal cells are scattered in the center of the body posterior to the eyespots. Living miracidia measure 0.068–0.108 × 0.036–0.052 mm.

Miracidial penetration into the molluscan host has been observed by several workers, notably Thomas (1883) in *Fasciola hepatica,* Barlow (1925) in *Fasciolopsis buski,* Bennett (1936) in *Cotylophoron cotylophorum,* Rees (1940) in *Parorchis acanthus* and Goodchild (1948) in *Gorgodera amplicava.* Barlow suggested that the apical gland secreted an "erosive fluid" while Goodchild considered it to secrete an adhesive substance. Several authors have observed droplets of fluid at the pores of the "penetration" glands and have assigned penetration functions to the secretions from these glands. Bennett pointed out that as transformation of the miracidium took place, it gradually changed shape and a very thin cuticula was formed around the outside of the body, but he did not give its origin.

In order to study the process of pentration and transformation of the miracidium into the sporocysts, an excised gill from a small uninfected *P. abditum* was added to a drop of water containing several miracidia on a slide. The actively swimming miracidia came into contact with the gill many times before they started to penetrate into the gill tissue. No attraction to the tissue was evident. The process of penetration was observed under high magnification of a compound microscope.

When penetrating into the gill, initially there is a rotatory movement combined with extreme prolongations of the anterior end of the larva, followed by progressive swelling from anterior to posterior, which draws the miracidium into the tissue. After approximately 15 minutes, rotary movements cease and the miracidium begins a rhythmical contraction and elongation of the body. Four small distinct droplets of granular material (Fig. 11) are extruded from the pores of the "penetration" glands. These pores are not symmetrically arranged although they each open in the anterior space between the first tier of ciliary plates. On the left of the miradicium one pore opens in the space between the dorso-lateral plates, while the other opens between the dorso-lateral and the lateral plates. On the right side of the body, however, one of the pores opens between the two ventro-laterals and the other between the ventro-lateral and lateral plates. The droplets coalesce into two droplets (Fig. 12). At this time granular material lighter in color and more fluid in consistency begins to flow from pores on the terrebratorium draining the apical gland. This material appears to be histolytic in function since there is a progressive breakdown and liquefaction of the clam tissue anterior to the miracidium. As additional granular material is extruded from the pores of the "penetration glands," the droplets fuse into an apical cap covering the anterior end. It could not be clearly observed if the secretion of the apical gland was pushed ahead of the forming cap or if it mixed with the cap material. At least some of the apical gland secretion stays anterior to the apical cap. As the apical cap becomes more extensive (Fig. 13), the sporocyst begins to extend into it from the miracidial covering by way of the terrebratorium.

The process of sporocyst emergence (Figs. 14–18) requires over three hours. During this time the apical cap becomes increasingly thicker until it extends 0.015

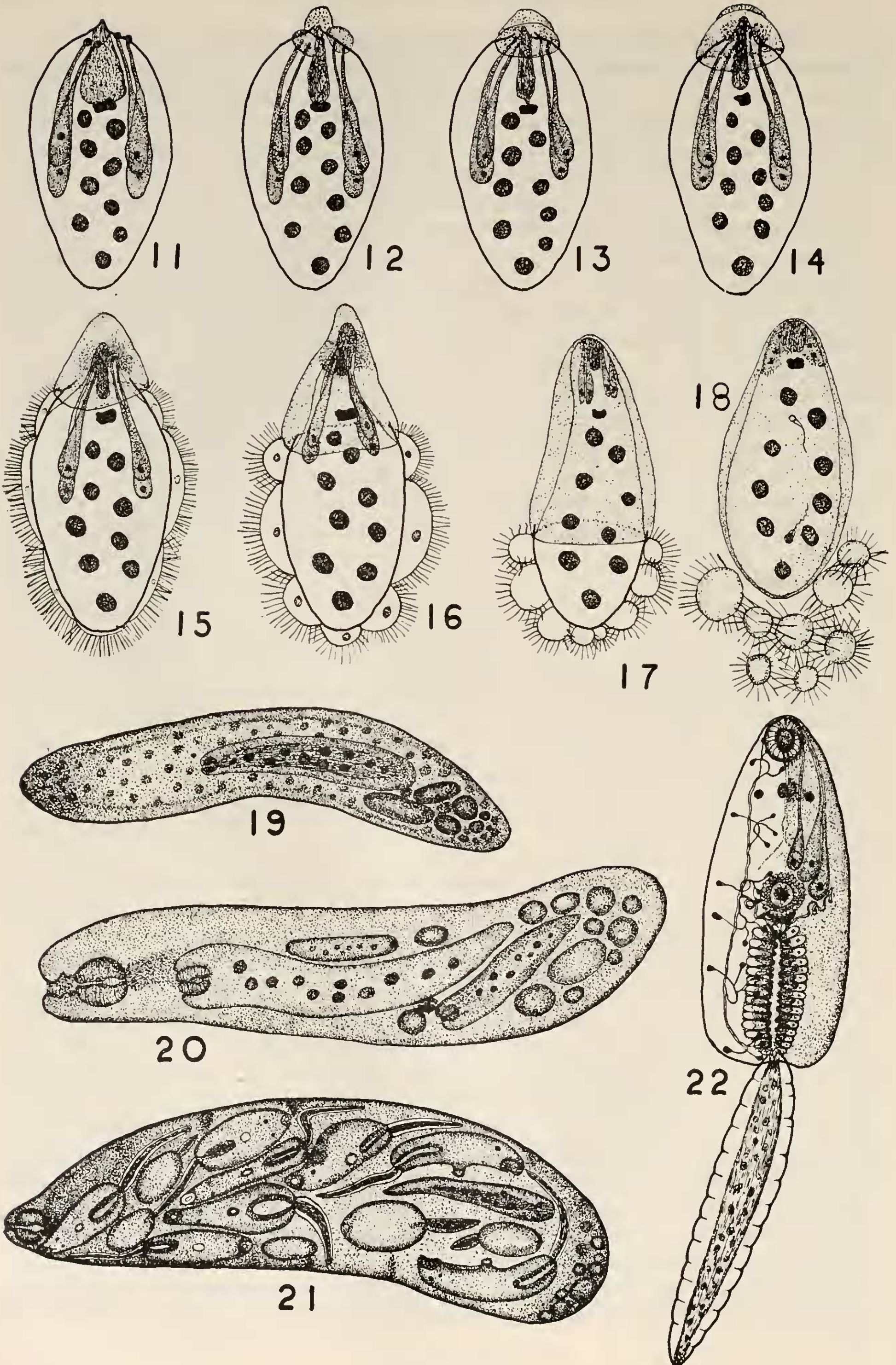


PLATE II

mm. anterior to the miracidium. The sporocyst gradually emerges into the apical cap material from the miracidial covering by a rhythmical series of anterior extensions and expansions. It is always covered by either the miracidial covering or the cap material since the latter gradually extends more posteriorly. As the granular material extends posteriad it appears to force the epidermal plate cells ahead of it. The epidermal cells become quite evident, each with a well defined nucleus. When the cap material covers about three-fourths of the emerging sporocyst, the epidermal cells are almost spherical and their cilia are perpendicular to the cell membrane and are still actively beating. A similar appearance of epidermal plate cells was described by Thomas (1883) and by Barlow (1925). Three hours after the sporocyst begins to emerge, the cap material completely covers the body of the sporocyst. This cuticle is more evident on the sides of the sporocyst than it is on the ends, being twice as thick on the former as on the latter. As the cuticle is fully formed, the rounded epidermal cells break away and are moved about by their beating cilia.

The apical gland thus appears to secrete a histolytic substance which aids in penetration and the "penetration" glands do not seem to aid in penetration other than perhaps passively by filling the space eroded by the material from the apical gland. Since the "penetration" glands do secrete material which forms a cuticle for the sporocyst, it would be more correct to call these "cuticle-producing" glands and assign the penetration function to the apical gland.

Sporocysts:

Newly formed sporocysts measure 0.061–0.068 × 0.029–0.031 mm. Eyespots are still contiguous and the apical gland and cuticle-forming gland remnants are confined to the anterior end of the sporocysts.

Additional development of the sporocysts was followed mainly in infections in *P. abditum* but *M. partumeium* infections were followed for two weeks and development in the two clam hosts was parallel. Presumably the infection in *M. partumeium* will also develop to the cercarial stage, but limited numbers of small clams of this species did not allow further study of development.

Much of the variation observed in the following developmental stages is due to the fact that clams were left in containers with hatching miracidia to insure infection, and since miracidia continued to hatch for several weeks, superimposed infections were common.

Four days after infection, the smallest sporocyst observed measured 0.08 × 0.051 mm., developing in the gill of the clam. Two eyespots were present, one in the middle of the anterior end and one almost midlength on the side of the body. No evidence of a sucker was found. Nine developing germinal cells occupied most of

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Explanation of Plate II.

Figures 11–14. Penetrating miracidium, showing secretions from "penetration" and apical glands and formation of apical cap (cilia omitted).

Figures 15–18. Formation of cuticle by gradual posterior progression of cap material forcing ciliated epidermal cells away from new sporocyst.

Figure 19. Sporocyst (0.42 × 0.082 mm.).

Figure 20. Mother redia (0.60 × 0.120 mm.).

Figure 21. Daughter redia (0.090 × 0.25 mm.).

Figure 22. Cercaria (body 0.341 × 0.15 mm., tail, contracted, 0.30 × 0.063 mm.), ventral view. Detail shown on the left and excretory system on the right of body.

the body space. The two flame cells were situated diagonally in the middle of the body.

Seven days after infection, clams yielded several sporocysts that measured 0.108–0.136 × 0.035–0.08 mm., with pigment sometimes diffuse but usually present as distinct eyespots, variable as to their location.

Eighteen days after infection, sporocysts measured 0.103–0.274 × 0.047–0.072 mm. In all sporocysts at this stage of development, eyespots were still present, and two flame cells were easily seen, one posterior and one anterior. Usually one germinal ball (sometimes two) had increased in size, and one was usually three or four times as large as the other germ balls.

By the 25th day the sporocysts were widely distributed in the tissues of the clam, being present in the gills, foot, digestive gland and mantle. These sporocysts ranged in size from 0.244–0.494 × 0.072–0.108 mm. At this stage eyespot pigment was usually diffuse or absent. An identifiable redia, with its conspicuous globular sucker, was usually present in each sporocyst. By the time the infection was 33 days old, rediae could be found in the tissues of the clam but their escape from sporocysts was not observed. Sporocysts (Fig. 19) increased very little in size beyond 0.50 × 0.12 mm.

At this stage, rediae surpassed the sporocysts in size, varying from 0.288 × 0.057 to 0.63 × 0.075 mm. The redial sucker is large, reaching 0.055 × 0.048 mm. The mother rediae (Fig. 20) are morphologically the same as the daughter rediae but their germinal cells give rise only to rediae and not to cercariae. Mother rediae usually contained several developing rediae, one of which was typically larger than the other.

In infections from 40 to 50 days old, sporocysts appeared to be absent while mother rediae with a maximum size of 0.73 × 0.094 mm. were still producing daughter rediae. There appeared to be only one generation of mother rediae, since daughter rediae began to differentiate identifiable cercariae when the infection was 50 days old.

Mature daughter rediae (Fig. 21) are elongate, thin-walled sacs without locomotory processes. The sucker is spherical, 0.04–0.057 mm.; the intestine reduced and inconspicuous. The birth pore is just anterior and lateral to the sucker, not clearly visible, but cercariae were observed escaping, one by one from the pore.

Twelve cercariae with definite eyespots were the maximum number observed in any redia. Combinations of developing cercariae with eyespots, and germinal masses numbered up to 18. Rediae containing eyed-cercariae measured 0.52–1.16 × 0.12–0.30 mm. (average size 0.87 × 0.20 mm.; average number of eyed-cercariae 7). Occasional rediae contained eyed-cercariae, germinal masses and daughter rediae. Rediae were also observed containing germ balls and daughter rediae. This would indicate that asexual multiplication is a continuous process and possibly continues for the life of the infection. Flame cells were four in number and appeared to be paired into two homologous systems, each with an anterior and a posterior flame cell. However, the ducts were difficult to distinguish.

Cercaria:

The cercaria is an ophthalmoxiphidiocercaria (Fig. 22), ellipsoidal in outline, from two to three times as long as wide, slightly depressed dorso-ventrally with

unarmed cuticle. Anterior sucker (0.047–0.05 mm. in diameter) equal to, or slightly larger than, ventral sucker. A stylet (Fig. 3) is present in the dorsal lip of the oral sucker, oriented at approximately a 45°–60° angle from longitudinal axis of the body. Stylet quite constant in size within each of the two populations, 0.023–0.024 mm. in cercariae from Coonamessett River and 0.021–0.0235 mm. in cercariae from Quashnet River. Stylet with lateral projections curved slightly upwards, about one-fourth the length of stylet from anterior end. Ventral sucker (0.042–0.048 mm. in diameter) located at about middle of the body, pedunculate, external margin bearing numerous serrate papillae. Prepharynx short; pharynx globular, 0.019–0.020 mm. in diameter, located mediad or slightly anteriad to the eyespots. Esophagus two to three times as long as the pharynx, bifurcation of the intestine just anterior to the midlength of the body. Caeca incompletely developed, usually reaching latero-posteriorly only to anterior edge of ventral sucker. Nervous system composed of a transverse band at the level of the pharynx with fibers extending to the eyespots. Eyespots well developed, brownish black. Three pairs of non-lobed penetration glands lie lateral and anterior to the ventral sucker. Each of the anterior pair of glands is drained by a duct which runs anteriad between the eyespots, while each of the two posterior pairs of glands has a duct which extends side by side anteriad between the body and the eyespots. Posterior and somewhat dorsal to the ventral sucker are the primordia of the genital organs abutting against the anterior end of the excretory bladder. Cystogenous glands appear to be absent and since no cyst is formed in caddis fly larvae, this might be expected.

The sac-shaped excretory bladder extends anteriorly almost to the ventral sucker. The wall of the bladder is thick, composed of numerous cells. Anteriorly two excretory canals enter laterally, proceeding along a sinuous path, from a point about mid-level to the ventral sucker where the posterior and anterior excretory ducts join. Ascending and descending ducts each drain three groups of flame cells: each group composed of four flame cells. The flame cell pattern is thus $2 [(4 + 4 + 4) + (4 + 4 + 4)]$.

The tail is attached slightly ventrally and is variable in length. Usually it is a little longer than the body but it can be extended to over twice the length of the latter. When fully contracted, it is shorter than the body but it is never as wide as the body, thus differing from the cercaria of *A. isoporum* as described by Looss (1894) and by Dollfus (1949).

Measurements of the tails of several cercaria killed by pipetting them into hot formalin solution ranged from 0.20–0.34 mm. in length and 0.036–0.042 mm. in width. Both Looss and Dollfus pointed out that the tail of the cercaria of *A. isoporum* possessed an inner medullary portion, containing the nuclei, and a clear outer transparent cortical zone. The tail of the the cercaria of *A. alloneotenicum* also has a medullary and a cortical layer very similar to *A. isoporum*.

Measurements of both the body and the tail of the cercariae are extremely variable in living as well as in preserved specimens. No method of killing and fixing the cercaria was found which gave consistent results, so that morphological features such as sucker size, stylet shape and size, and size and extent of the excretory bladder are more reliable descriptive characteristics.

A single precocious cercaria which was 0.81×0.30 mm., with a tail 0.17 mm. long, was present in a redia 0.968 mm. long. Immature gonads were clearly de-

fined. The stylet was absent in this precocious cercaria. Since the stylet was observed to aid in the escape of normal cercariae from rediae, this might account for the retention of the cercaria. The increase in size and development of gonads while still in the clam host is surprising.

Juvenile worms:

No cysts are formed although occasionally young worms became isolated in the gills of the caddis fly larvae and thus appeared cyst-like. Such worms seem to be prevented from reaching the haemocoel by the accumulation of detached tracheal vessels in the proximal part of the gill. Isolated worms typically cause the deposition of dark brown pigment by the larvae, which is a common response to any mechanical injury at any place in their bodies.

Experimental infections of caddis fly larvae isolated with individual clams liberating *A. alloneotenicum* cercariae resulted in the presence of numerous juvenile worms. One hundred caddis fly larvae were brought into the laboratory from sources thought to be free of infection; 50 were dissected and found to be negative, 25 were used in infection experiments and the remaining 25 were kept as controls and found to be negative upon dissection at the conclusion of the experiment.

In infections up to a week old, the worms varied from 0.27–0.33 × 0.13–0.17 mm. The pharynx was 0.02–0.021 mm. in diameter; the oral sucker 0.057–0.058 mm. and the ventral sucker 0.047–0.050 mm. In the larger specimens the genital primordium had begun to differentiate into identifiable reproductive organs. In later stages the testes had developed more rapidly than the ovary, similar to the development of these structures in other trematodes.

Eggs are present in infections 24 days old, but are few in number for an additional 14 days during which the worms continue to increase in size. When eggs are first produced, the worms are usually 1.5 × 0.62 mm. in size but the number of worms present in the larvae influences this size as well as the ultimate sizes.

Discussion

Peters (1957) reviewed the genus *Allocreadium* and emended the generic diagnosis, retaining 16 of the 31 species described in the genus. He further listed 5 as *species dubiae* and transferred the remaining 10 species to other genera or left them as *species inquirendae* because of inadequate descriptions.

A. alloneotenicum conforms to the genus *Allocreadium* as emended by Peters (1957). It can be separated from the other species in the genus, however, by the extreme anterior position of the ventral sucker (within the anterior one-sixth of the body), and in the position of the testes (within the first half of the body). It specifically differs from *A. ictaluri* Pearse 1924, and *A. pseudotritoni* Rankin 1937 in lacking vitellaria in the forebody; by ventral sucker being larger than the oral sucker it differs from *A. handiai* Pande 1937, *A. nicolli* Pande 1938a, *A. kosia* Pande 1938a, and *A. mahaseri* Pande 1938b. *A. alloneotenicum* also differs from *A. transversale* (Rudolphi 1802) Szidat 1939, *A. schizothorcis* Pande 1938b, *A. lobatum* Wallin 1909, and *A. hasu* Ozaki 1926 in having the ventral sucker well within the anterior fourth of the body, and in the shape and position of the gonads. It differs from the type species *A. isoporum* (Looss 1894), and from *A. nemachilus* Kaw 1950 and *A. thapari* Gupta 1950 in the size and distribution of the vitelline

follicles, the position of the cirrus sac, extent of the uterus and number and size of eggs.

The original descriptions of *A. markewitchi* Koval 1949 and *A. dogieli* Koval 1950 were not available for comparison but from the description of these species in Markevich (1952), it is apparent that *A. alloneotenicum* is distinct from these species.

A. alloneotenicum corresponds most closely to *A. neotenicum* Peters 1957. It differs in the shape of the body (always at least twice as long as wide), the posterior extent of the vitellaria and caeca, in the extent of the excretory bladder (which only reaches mid-level of the posterior testis instead of to under the anterior testis), and in the relative position of the ovary complex and the cirrus sac. *A. neotenicum* and *A. alloneotenicum* are unique in that they are the only two species within the genus known which apparently develop to sexual maturity in insects.

The clam, *P. abditum,* from Coonamessett also contained an infection of *Crepidostomum* sp. The cercariae from this infection were also ophthalmoxiphidiocercariae developing from rediae. They encysted both in nature and experimentally in the amphipod, *Gammarus* sp. *Crepidostomum* infections could be differentiated in the redial stages by the larger number of cercariae (usually approximately 36 being present) as well as by morphological differences.

The *Crepidostomum* cercariae possess a slightly smaller stylet, 0.017–0.019 mm., which has more of a median keel and slightly different lateral projections. They also possess 44–48 clearly defined cystogenous glands, 12–14 anterior and 32–34 posterior to the ventral sucker. The three pairs of penetration glands tend to be lobed. The excretory bladder does not extend as far anteriorly and the genital primordium is more extensive.

No fish were collected from this pool during the period November, 1956 to April, 1957. Attempts to infect various fish, including *Eucalia inconstans, Fundulus heteroclitus,* and *Salvelinus fontinalis,* with infected amphipods yielded only a limited number of juvenile *Crepidostomum* from the trout.

Infection experiments using the same three species of fish, feeding them caddis fly larvae known to be infected with juvenile *A. alloneotenicum,* were all negative. The worms were digested with the caddis fly larvae and portions of both could be recovered on the second day from the posterior portion of the gut of the fish. It might be possible that eggs would remain viable after passage through the intestine of a fish, but this was not investigated.

It appears, from the large number of eggs produced by the adult worms (up to 1200 eggs being recovered from a larva containing a single worm), that the infection in caddis fly larvae is the normal one for this species of *Allocreadium.* Infected larvae are never as active as uninfected ones; they are usually smaller and their cases show signs of neglect. The presence of numerous eggs throughout the body of the caddis fly larvae, including the appendages and the head capsule, plus the erosion and decrease in numbers and size of the fat-bodies make it extremely unlikely that an infected larva is ever able to pupate and reach adulthood. The recovery of the remains of dead larvae, still within the cases, enclosing empty egg shells of *A. alloneotenicum,* substantiates this view.

Peters (1955, 1957) stated that *Allocreadium neotenicum* from aquatic beetles from Michigan (possibly identical with species found by Crawford (1940) in aquatic beetles from Colorado) was a progenetic form that did not require a

vertebrate host in order to complete its life cycle. He presented ecological evidence which supported his supposition. Buttner (1950, 1955) reviewed the progenetic trematodes and proposed four degrees of development of this characteristic. Peters (1957) added *A. neotenicum* to the fourth group of Buttner, that is, to the group in which the development of the gonads, the genital activity and the fecundity rivals that of the true adult. The example of this group cited by Buttner was *Paralepoderma brumpti,* a plagiorchid. *A. alloneotenicum* should be added to this group also. Both of these species of *Allocreadium* differ from the example cited by Buttner, however, since they develop to maturity in invertebrate rather than in vertebrate hosts.

The presence of ophthalmoxiphidiocercariae developing from rediae in sphaeriid clams in *A. alloneotenicum* supports the systematic scheme proposed by Dollfus (1949). Seitner's work (1951) should be re-investigated in the light of the results of the present study before final acceptance of the scheme of Dollfus. The controlled experiments on the life-history of *A. alloneotenicum,* supporting the morphological and ecological data presented by Looss (1894) and Dollfus (1949) for *A. isoporum* and by Peters (1957) for *A. neotenicum,* indicate a close relationship of the genera *Allocreadium, Crepidostomum,* and *Megalonia.* They all have ophthalmoxiphidiocercariae developing in rediae from sphaeriid bivalves thus forming a natural group.

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Summary

Allocreadium alloneotenicum sp. nov. is described from the haemocoel of *Limnéphilus* sp., caddis fly larvae, from Cape Cod, Massachusetts. The life-cycle is demonstrated both in natural and experimental infections. The normal clam host is *Pisidium abditum.* Miracidia hatch in the debris from dead larvae or after ingestion and passage in the feces of the snail, *Aplexa hypnorum.* The process of miracidial peneration was observed. Secretions from the apical gland are histolytic in action, facilitating penetration, while the "penetration" glands produce the cuticula of the sporocyst. Sporocysts give rise to one generation of mother rediae which in turn liberate daughter rediae. Daughter rediae give rise to ophthalmoxiphidiocercariae and also produce occasional rediae. The cercariae penetrate caddis fly larvae (as many as 25 of them being found in natural infections). They mature in the haemocoel and a single worm was found to have laid 1200 eggs. Experimental infections of fish with infected caddis fly larvae were negative.

LITERATURE CITED

Barlow, C. H., 1925. The life history of the human intestinal fluke, *Fasciolopsis buski* Lankester. *Amer. J. Hyg., Monogr. Ser.,* **4**: 1–98.

Bennett, H. J., 1936. The life history of *Cotylophoron cotylophorum,* a trematode from ruminants. *Illinois Biol. Monogr.,* **14** (4) : 1–119.

Buttner, A., 1950. La progénèse chez les trématodes digénétiques. Sa signification. Ses manifestations. Contributions à l'étude de son déterminisme. *Ann. Parasitol.*, **25** (5–6) : 376–434.

Buttner, A., 1955. Les distomes progénètiques sont-ils des pré-adultes ou des adultes véritables? Valeur évolutive de la progénèses chez les Digenea. *C. R. Soc. Biol.*, **169**: 267–272.

Crawford, W. W., 1940. An unusual case of a sexually mature trematode from the body cavity of a diving beetle. *J. Parasitol.*, 32, Suppl.: 32.

Dollfus, R. Ph., 1949. Sur une cercaire ophthalmoxiphidiocerque *Cercaria isopori* A. Looss 1894 et sur la délimitation des Allocreadioidea. *Ann. Parasitol.*, **24**: 424–435.

Goodchild, C. G., 1948. Additional observations on the bionomics and life history of *Gorgodera amplicava* Looss, 1899 (Trematoda, Gorgoderidae). *J. Parasitol.*, **34** (5) : 407–427.

Gupta, S. P., 1950. On a new trematode, *Allocreadium thapari* n. sp. of the sub-family Allocreadiinae Looss, 1899 from the intestine of a fresh-water fish, *Rita rita* (Ham). *Indian J. Helminth.*, **2**: 17–22.

Kaw, B. L., 1950. Studies in helminthology: Helminth parasites of Kashmir. Part I. Trematoda. *Indian J. Helminth.*, **2**: 67–126.

Looss, A., 1894. Die Distomen unserer Fische und fröche neue Untersuchungen über Bau und Entwickelung des Distomenkörpers. *Biblioth. Zool. Stuttgart*, **6** (16) : 1–296.

Lynch, J. E., 1933. The miracidium of *Heronimus chelydrae* MacCallum. *Quart. J. Micros. Sci.*, **76**: 13–33.

Markevich, A. P., 1952. Parasite fauna of fresh-water fish of Ukrainian SSR. Kiev.

Mathias, P., 1937. Cycle évolutif d'un trématode de la famille *Allocreadiidae* Stossich (*Allocreadium angusticolle* (Hausmann). *C. R. Acad. Sci. Paris*, **205** (15) : 626–628.

Ozaki, Y., 1926. On some new species of trematodes of fresh water fish from Japan (Preliminary report.). *Dobuts. Zasshi, Toyko*, **38**: 124–130. (In Japanese).

Pande, B. P., 1937. Morphology and relationships of a new digenetic trematode from an Indian freshwater fish, *Ophiocephalus punctatus*. *Ann. Mag. Nat. Hist., Ser. 10*, **20**: 415–421.

Pande, B. P., 1938a. The trematode genus *Allocreadium* in north Indian freshwater fishes. *Proc. Indian Acad. Sci.*, **7** (B) : 54–60.

Pande, B. P., 1938b. On two new trematodes from Indian cyprinoid fishes with remarks on the genus *Allocreadium* Looss. *Proc. Nat. Acad. Sci., India*, **8**: 110–115.

Pearse, A. S., 1924. Observations on parasitic worms from Wisconsin fishes. *Trans. Wisconsin Acad. Sci.*, **21**: 147–160.

Peters, L. E., 1955. Morphology of the adult and the miracidium of a progenetic species of *Allocreadium* from water beetles of the family Dytiscidae. *J. Parasitol.*, **41**, Suppl.: 36.

Peters, L. E., 1957. An analysis of the trematode genus *Allocreadium* Looss with the description of *Allocreadium neotenicum* sp. nov. from water beetles. *J. Parasitol.*, **43**: 136–142.

Rankin, J. S., 1937. New helminths from North Carolina salamanders. *J. Parasitol.*, **23**: 29–42.

Rees, F. G., 1940. Studies on the germ-cell cycle of the digenetic trematode *Parorchis acanthus* Nicoll. Part II. Structure of the miracidium and germinal development in the larval stages. *J. Parasitol.*, **32**: 372–391.

Seitner, P. G., 1951. The life cycle of *Allocreadium ictaluri* Pearse, 1924 (Trematoda: Digenea). *J. Parasitol.*, **37**: 223–244.

Szidat, L., 1939. Über *Allocreadium transversale* Rud. 1802 aus *Misgurnus fossilis* L. *Zeitschr. f. Parasitenk.*, **10**: 468–475.

Thomas, A. P., 1883. The life history of the liver fluke. *Quart. J. Microsc. Soc.*, **23**: 90–133.

Wallin, I. E., 1909. A new species of the trematode genus *Allocreadium*. *Trans. Amer. Micros. Soc.*, **29**: 50–66.